



Research Article

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The study of the antimicrobial activity of *Carduus crispus* extracts

Omirbaeva A. E.¹, Datkhaev U. M.¹, Gladukh. Ie. V.², Iudina Iu.V.^{2*}, Strilets O. P.² and Strelnikov L. S.²

¹Asfendiarovs' Kazakh National Medical University, Kazakh Republic, Almaty, Ukraine

²National University of Pharmacy, Ukraine, Kharkov

ABSTRACT

Given article deals with the results of the study of antimicrobial activity and microbiological purity of *Carduus crispus* (C.C.) extracts. It has been found that 40% C.C. extract exhibits antimicrobial activity against all the bacterial culture: Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, as well as gram-negative *Escherichia coli*. 90% C.C. extract exhibits the same antimicrobial activity against all the bacterial culture: Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, as well as gram-negative *Escherichia coli*. It was established experimentally that the extract C.C. 90% has a higher degree of antimicrobial activity than the extract C.C. 40%. No activity was observed with respect to the culture of yeast-like fungus *Candida albicans*. Both samples meet the requirements of the Pharmacopeia of Ukraine in terms of "microbiological purity of non-sterile drugs."

Key words: extract, *Carduus crispus*, antimicrobial activity, microbiological purity.

INTRODUCTION

The development of the pharmaceutical industry in the Kazakh Republic appropriate to conduct through the development of phytochemical production as the country poses unique resource base of medicinal plants, a significant scientific and technical potential in the field of chemistry, medicine and pharmacy, and traditional orientation of domestic manufacturers on processing of medicinal plants. In this case, one of the main priorities for the development of the domestic pharmaceutical industry is the development and introduction of original domestic substances based on medicinal plants and drugs on their basis.

Carduus crispus grows in the southern regions of Kazakhstan and its raw material reserves are estimated at thousands of tons per year. So the implementation of this raw materials in the pharmaceutical technology is promising and meets the modern challenges of the pharmaceutical industry of the Kazakhstan Republic.

We have obtained 40% and 90% ethanol extracts of *Carduus crispus* (C.C.), which were tested for antimicrobial activity and microbiological purity.

EXPERIMENTAL SECTION

Objects and methods of research.

The studies of antimicrobial activity of the extracts was performed at the Department of Biotechnology of the National University of Pharmacy. For the analysis were obtained samples of extracts:

№1. 40% extract of C.C.;

№2. 90% extract of C.C.

The antimicrobial activity of the extracts samples was studied in vitro by the method of diffusion in agar well which is based on the ability of active substances to diffuse into the agar previously seeded by microorganisms cultures [1]. All studies were conducted in strict aseptic conditions, using a laminar box (cabinet of biological safety AS2-4E1 "Esco", Indonesia).

As a test cultures using microorganisms from the American Typical Culture Collection (ATCC - American Typical Culture Collection): Gram-positive bacteria *Staphylococcus aureus* ATCC 25293, *Bacillus subtilis* spore culture ATCC 6633, a Gram-negative *Escherichia coli* culture ATCC 25922. antifungal activity was determined with respect to the yeast-like fungi *Candida albicans* ATCC 885-653 [2].

Index of antimicrobial activity is the size of the delay growth of test microorganisms zones that is formed in agar medium on Petri dishes. The diameter of the zones of growth inhibition considering the wells diameter was measured with accuracy of 1 mm, while focused on the complete absence of visible growth.

For research was used DSA suspension of bacterial microorganisms in saline, and a two-day culture of yeasts. Microbial load was 1×10^7 of microbial colony forming units in 1 ml culture medium (CFU / ml) [1].

In Petri dishes mounted on a horizontal plane were placed 10 ml of uninfected "hungry" AGV agar (for the upper layer when using bacterial cultures used the meat-peptone agar (MPA), working with yeast-like fungi - Saburo agar). After solidification of this agar layer on its surface at an equal distance from each other and from the edge of the cup were placed a sterile steel cylinders (height $10,0 \pm 0,1$ mm, outer diameter $8,0 \pm 0,1$ mm) and was poured melted and chilled to $45-48^\circ\text{C}$ upper agar layer with cultures of microorganisms in an amount of 15 ml. After cooling and solidification of the upper layer of the culture medium cylinders was removed with sterile forceps and in the formed wells were poured studied extracts samples (0.25-0.3 ml) [1].

In parallel was conducted study with solvents of samples №1 and №2 - ethyl alcohol 40% and 90%, respectively.

Petri dishes with crops placed in an incubator - bacterial cultures at $32,5 \pm 2,5^\circ\text{C}$ for 18-24 h, culture yeasts at $22,5 \pm 2,5^\circ\text{C}$ for 48 h. The diameters of the zones of inhibition of microbial growth characterize the antimicrobial activity of the samples.

When studying microbiological purity of samples was used method of extracts the State Pharmacopoeia of Ukraine (1.4, p. 5.1.4 - microbiological purity of non-sterile medicines, p. 171), which allows objectively evaluate the quality characteristics of the samples on the basis of experimentally obtained statistically processed results [2, 3]. Estimation of microbiological contamination degree of the drug include the identification the total number of aerobic mesophilic bacteria (TAMC) and total yeasts and molds (TYMC) 1 g extracts, establishing the absence of bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. In 1 g of non-aqueous medicinal products for oral and rectal administration the total number of aerobic microorganisms (TAMC) may be not more than 103 CFU (colony forming units); the total number of yeasts and molds (TYMC) not more than 102 CFU [3]. To check the suitability of determination methods of total viable aerobic microorganisms as a test-strains was used the following bacteria from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 [3].

According with the requirements of the Ukrainian Pharmacopoeia was used following dense and liquid nutrient medium: Casein soy agar (to determine the number of live bacteria), Sabouraud dextrose agar (to determine the number of fungi), soybean casein broth (preincubation for in determining the presence of certain microorganisms), manitn-saline agar (for identification of bacteria *Staphylococcus aureus*), cetrimide agar (to identify *Pseudomonas aeruginosa*), Mac Conkey agar (for bacteria detection of *Escherichia coli*).

RESULTS AND DISCUSSION

The diameter of the microorganism growth characterizes the antimicrobial activity of the experimental samples as follows:

- absence of growth inhibition zones of the microorganisms around the wells, as well as growth inhibition zone diameter less than 10 mm was evaluated as the insensitivity of microorganisms to the extract samples introduced into the wells;
- the zone of growth inhibition diameter of 11-15 mm were evaluated as weak sensitivity of the culture to the active ingredients of the samples extracts;
- the zone of growth inhibition diameter 15-25 mm - strain sensitive to the sample;

- the zone of growth inhibition, with a diameter more than 25 mm, testified to the high sensitivity of microorganisms to the sample extracts.

The result of studies of the extracts samples antimicrobial activity for the various cultures of microorganisms shown in table 1.

Table 1 Antimicrobial activity of the extracts samples

Sample Specimen	Cultures of microorganisms			
	<i>S.aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>C. albicans</i>
	Zone of microorganisms growth delay diameter, mm			
Control (ethanol 40%)	-	-	-	-
Control (Ethanol 90%)	-	-	-	-
№1 40% extract of C.C.	20-21	16-17	18-19	-
№2 90% extract of C.C.	22-23	19-20	21-22	-

“ - “ – no zone of microorganisms growth inhibition.

The data obtained experimentally and shown in Table 1, showed that control (ethyl alcohol 40%) and control (90% ethyl alcohol) do not have antimicrobial activity against all used microorganisms.

Sample №1 (C.C.extract 40%) exhibits antimicrobial activity against all the bacterial culture: Gram-positive *Staphylococcus aureus* - 20-21 mm, *Bacillus subtilis* - 16-17 mm, as well as gram-negative *Escherichia coli* - 18-19 mm. With respect to the yeast-like fungus *Candida albicans* activity was not observed.

Sample №2 (C.C. extract 90%) exhibits the same antimicrobial activity against all the bacterial culture: Gram-positive *Staphylococcus aureus* - 22-23 mm, *Bacillus subtilis* - 19-20 mm, as well as gram-negative *Escherichia coli* - 21-22 mm. With respect to the culture of yeast-like fungus *Candida albicans* activity was not observed.

Should be noted that samples extracts №1 and №2 have an average degree of antimicrobial activity (diameter of zones microorganisms growth inhibition 15-25 mm) with respect to the bacterial cultures of microorganisms. Sample №2 is more active in comparison with the sample №1: towards *Staphylococcus aureus* - 22-23 mm and 20-21 mm, respectively, to the *Bacillus subtilis* - 19-20 mm and 16-17 mm, respectively, for *Escherichia coli* - 21-22 mm (№2) and 18-19 mm (№1).

When determining the microbiological purity of the analyzed extracts in order to prevent errors in the evaluation of results, preliminary studies it was found that all the samples of the extracts have antimicrobial activity. For neutralization of the antimicrobial action diluted extracts 1:10 were prepared by adding of a buffer solution with sodium chloride and peptone pH 7.0. After dilution of the extracts (1:10) antimicrobial activity in all of the samples was not observed.

For analysis were taken 2.0 g of the extract test sample was added to the buffer solution of sodium chloride and peptone pH 7.0 to a final volume 20 ml (1:10 dilution). In a Petri dish 9 cm in diameter was added 15 ml of casein-soya agar or Sabouraud-dextrose agar at a temperature from 45 to 50 ° C, culture media was allowed to cool.

1 ml of the test dilutions (1:10) were added into tubes containing 4 ml of melted and cooled to a temperature not more than 45 ° C agar medium. The tube contents were rapidly mixed and transferred into a Petri dish with the prepared first layer of the nutrient medium. By a quick shake of the Petri dishes evenly distributed top layer of the medium. For each dilution were prepared three Petri dishes for each culture medium.

Dishes with casein-soya agar were incubated at 30-35 ° C 5 days dishes with Sabour and dextrose agar were incubated at 20-25 ° C for 7 days. For each nutrient medium was calculated arithmetic mean value the number of colonies, and was determined the number of CFU per gram of medicament.

Incubation of prepared extracts samples (1:10 dilution) for manitno-salt agar (temperature 30-35°S - 72 hours), cetrimid agar (temperature 30-35 ° C - 72 hours) and MacConkey agar (30-35 ° C - 72 hours) showed the absence of colonies, which corresponds to the result "no bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* in 1 g of the test samples of extracts."

The results of microbiological purity of the extracts samples studies are shown in the table 2.

Table 2 The results of the microbial purity control of C.C. extracts

Sample	Amount of sample	Dilution	The total number of microorganisms in 1 g of the extract		Microorganisms		
			bacteria (TAMC) CFU / g	fungi (TYMC) CFU / g	<i>E. coli</i>	<i>Staph. aureus</i>	<i>Ps. aeruginosa</i>
№1 40% extract of C.C.	2,0 g	1:10	<10	<10	no growth	no growth	no growth
№2 90% extract of C.C.	2,0 g	1:10	10	<10	no growth	no growth	no growth

Thus, it is experimentally proved that the samples of extracts №1 and №2 did not reveal the presence of bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Found that the total number of fungi (TYMC) is less than 10 CFUs / g in all extracts samples (№1 C.C. extract 40% №2 C.C. extract 90%).

The number of bacteria (TAMC) in 1 g of the test samples of extracts is 10 CFUs / g for sample №2 (C.C. extract 90%).

For sample №1 (C.C. extract 40%) number of bacteria (TAMC) in 1 g of the test samples of extracts is less than 10 CFUs / g.

The results show that extracts samples №1 and №2 meet the requirements of the Pharmacopeia of Ukraine in terms of "microbiological purity of non-sterile drugs."

CONCLUSION

Based on these studies can draw conclusions that the C.C. extracts have moderate antimicrobial activity against to the bacterial cultures of microorganisms. The 90% extract is more active as compared to 40% extract: with respect to *Staphylococcus aureus* - 22-23 mm and 20-21 mm, respectively, to the *Bacillus subtilis* - 19-20 mm and 16-17 mm, respectively, in *Escherichia coli* - 21-22 mm and 18-19 mm. Both samples meet the requirements of the Pharmacopeia of Ukraine in terms of "microbiological purity of non-sterile drugs." These extracts can be used to develop formulations with antimicrobial activity.

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